REMARKS

The claims in the case are claims 1-11. Claims 1 and 11 are the independent claims. In its broadest sense, each of these independent method claims relates to lowering egg cholesterol levels of laying fowl by administering polycosanol. The Examiner has rejected each of these independent claims on a theory of inherency and lack of novelty, based upon three references. Those references, Havens, Lane et al., and JP4-210560 each teach feeding laying fowl a diet that contains something which the Examiner urges has within it polycosanol. Havens teaches cabbage; Katta teaches rice bran; and, Lane teaches rice bran. From this, the Examiner leaps to the conclusion that in each instance the independent claims are anticipated because the compositions containing cabbage and/or rice bran would inherently lower cholesterol as they contain some amount of polycosanol. This conclusion, reached by the Examiner, on nothing more than a leap of faith is simply wrong. Each of the claims in independent form, i.e. 1 and 11, require "administering a cholesterol lowering effective amount". The Examiner relies on nothing but faith to urge that this claim limit is met. Respectfully, it is not; and therefore the references cannot anticipate. A discussion of each reference and its true teaching follows.

JP4-210560A Yamamoto. The Examiner states that the inventor in E1 claims rice bran as an agent for lowering egg yolk cholesterol and since dried bran contains wax esters, a source of polycosanol, it anticipates use of polycosanol to change the cholesterol content of yolk. With respect, a careful reading shows that the inventor did not use rice bran but fermented rice bran to produce an "intermediate feed" that was fed to chickens. Since the starting rice bran has been altered by fermentation, it is incorrect to conclude that there is any polycosanol in the intermediate feed.

US 5,091,195 Havens. Similar to above, the Examiner states that since cabbage contains polycosanol then it would inherently use polycosanol for the laying hen diet to lower cholesterol. However, the inventor teaches away from this assertion by stating in col. 2, lines 19-32 that a component in cabbage induces the production of cytochrome P-450 that "detoxifies" cholesterol and allows it to be used for energy purposes, resulting in less cholesterol for the egg. This mechanism is specifically claimed in his Claim 5.

US 5,578,584 Katta et al. With respect, the Examiner has misunderstood the role of rice bran in Table 10 of the above-referenced patent. The feed described in Table 10 is a basal feed that contains rice bran that was added to two groups of egg laying hens. One group received only the basal feed (placebo, col. 10, lines 23-42) and the other group received the basal feed plus galacto-oligosaccharides (col. 10, lines 44-4), the compounds that were the subject of the invention. The effect of the galacto-oligosaccharides was to lower egg yolk cholesterol. If the inventor thought rice bran had an effect on cholesterol lowering, he would not have used it as a basal feed. Once again this teaches away from the use of polycosanols as agents for lowering egg yolk cholesterol.

US 6,239,171 Lane et al. The purpose of this invention was to demonstrate that certain tocotrienols and tocotrienol-like compounds found in rice bran or its oil lower serum cholesterol and also can be used for the treatment of inflammatory conditions. Example 8 clearly shows that the wax fraction, which contains the polycosanols and phytosterols, do not plan a role in cholesterol reduction. Thus, rice bran wax (Protocol VII, col. 22, lines 57-62) was isolated and added to a placebo basal chick diet (Table IV, entry 1) to determine its effects on circulating cholesterol. The inventor concluded (col. 31, lines 25-26) that the wax fraction that contained the sterols (and the polycosanols) had no effect on circulating cholesterol.

The Examiner should also be aware that fiber has an impact on the cholesterol content of eggs. McNaughton reported that egg yolk cholesterol was significantly decreased when fiber from alfalfa meal, oats, sunflower meal, rice mill feed or wood shavings was fed to laying hens ("Effect of dietary fiber on egg yolk, liver, and plasma cholesterol concentrations of the laying hen, *J. Nutr.* 108:1842-1848, 1978 (copy enclosed)). The consumption of large amounts of cabbage would also be expected to be a good source of fiber. In summary, when gross diet feeding studies are performed which contain a source of fiber, attribution of a cholesterol lowering effect to one component is problematic unless that component is purified and added to the laying hen in a controlled trial.

There is simply no basis to conclude that these references properly interpreted in fact use polycosanols to lower cholesterol by administering an effective amount to lower cholesterol. Inherency must be based on evidence and facts not upon wild speculation. Purdue Pharma L.P. v. Faulding Inc., 56 U.S.P.Q.2d 1481, 1483 (Fed. Cir. 2000) (claim limitation is inherent if one skilled in the art can *immediately discern* the limitation at issue) (emphasis added); Tronzo v. Biomet, Inc., 47 U.S.P.Q.2d 1829, 1834 (Fed. Cir. 1998) ("In order for a disclosure to be inherent ... the missing descriptive matter must *necessarily be present* in the parent application's specification such that one skilled in the art would recognize such a disclosure.") (emphasis added); and Silvestri v. Grant, 181 U.S.P.Q. 706, 709 (1974) (inherent properties are redundant and add *no additional limitations* beyond those recited) (emphasis added).

As the recent Supreme Court KSR case teaches, references that teach away can be evidence of patentability. Here the Examiner cites references when taken together teach away from the use of polycosanols and the waxes from which they are derived for lowering circulating cholesterol and the cholesterol content of egg yolk. In this way it cannot be seen to be

anticipated (it's not inherent as demonstrated in the discussion above) and neither can it be obvious since the references teach directly contrary to the claimed process. Reconsideration and allowance is requested.

No fees or extensions of time are believed to be due in connection with this amendment; however, consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

EDMUND J. SEASE, Reg. No. 24,741
McKEE, VOORHEES & SEASE, P.L.C.
801 Grand Avenue, Suite 3200
Des Moines, Iowa 50309-2721
Phone No: (515) 288-3667
Fax No: (515) 288-1338
CUSTOMER NO: 22885

Attorneys of Record

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Effect of Dietary Fiber on Egg Yolk, Liver, and Plasma Cholesterol Concentrations of the Laying Hen

JAMES L. McNAUGHTON
U.S. Department of Agriculture, S.E.A., F. R.
South Central Poultry Research Laboratory,
Mississippi State, Mississippi 39762

ABSTRACT Two experiments were conducted to determine the effect of dietary fiber source and level on egg yolk, liver, and plasma cholesterol concentrations of White Leghorn laying hens. Initially, dietary fiber levels of 2.05, 4.41, 6.68, and 8.79% furnished mainly by sunflower meal were fed to laying hens for 140 days. In the second experiment, alfalfa meal, ground whole oats, sunflower meal, rice mill feed, or wood shavings was added to a corn-soybean meal basal diet to furnish 2.00% added crude fiber and fed to laying hens for 84 days. Yolk cholesterol decreased 4.39, 10.38, and 13.29% by feeding crude dietary fiber levels of 4.41, 6.68, and 8.79%, respectively, to hens as compared to a corn-soybean meal basal diet containing 2.05% crude fiber. Egg yolk cholesterol was significantly decreased by feeding alfalfa meal, oats, sunflower meal, rice mill feed, or wood shavings to laying hens when compared to yolk cholesterol of hens fed the basal diet. The greatest reduction in egg yolk cholesterol was found by feeding either oats or wood shavings. No significant differences were found in plasma cholesterol due to dietary fiber level. Plasma triglycerides decreased and liver cholesterol increased as dietary fiber level increased in diets fed to laying hens. When laying hens were fed alfalfa meal, oats, rice mill feed, or wood shavings, plasma cholesterol significantly decreased. Liver cholesterol increased when hens were fed either alfalfa meal or rice mill feed as the primary fiber source. J. Nutr. *108:* 1842–1848, 1978.

INDEXING KEY WORDS dietary fiber · cholesterol concentration · laying hens

Dietary fiber has been implicated in recent years as causing a reduction in serum and body cholesterol. Fiber, the nondigestible component of a diet, has been referred to as a natural hypocholesteremic agent. Balmer and Zilversmit (1) found that nondigestible components of an animal's diet have major influences on both plasma cholesterol concentrations and turnover, as well as fecal excretion.

Increasing dietary fiber has been shown to significantly decrease serum cholesterol and/or artery deposition of plaque in humans (2), rabbits (3), rat (4), chicks (5–6), turkeys (7), and laying hens (8–9). Efforts have been made by numerous researchers to lower egg yolk cholesterol. A few of these areas of research include drugs (10), dietary proteins (11), energy level or energy sources (12), vitamin A (13), ascorbic acid (14), and vanadium (15).

Turk and Barnett (16) found that alfalfa, when added to a corn-soy laying hen diet, was the most effective of the fiber sources tested for decreasing egg cholesterol with the least loss of egg size, feed efficiency, and egg production, while cellulose only

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slightly reduced egg cholesterol. Menge et al. (8) found that increasing the dietary fiber level from 4.1 to 17.7% with cellulose caused a reduction in serum cholesterol and an increase in egg yolk cholesterol. Story and Kritchevsky (17) found that cellulose bound an average of 1.4% of all the bile acids tested, whereas alfalfa bound 15.9%; thus, alfalfa was most successful in reducing bile acids. Husseini et al. (9) found that the mean blood serum and egg yolk cholesterol levels of commercial egg-type pullets were not affected by a diet containing 15% ground oats and 3% vegetable oil. Since vegetable oil (18) has been found to increase body cholesterol, there appear to be both hypo- and hypercholesteremic agents added to diets tested by Husseini et al. (9).

Fiber has been found to reduce body cholesterol of various animal species. However, relationships of dietary fiber to edible excretory products, such as eggs, have not been tested thoroughly with the exception of extremely high dietary fiber levels, which have been shown to affect production of laying hens. In studies that have been conducted, both hypo- and hypercholesteremic agents were added, thus nullifying the effect of both additives. Therefore, experiments were conducted to determine the effect of fiber source and level on yolk and body cholesterol of laying hens using natural fiber sources that might be added to practical laying hen diets.

PROCEDURE

A total of 792 White Leghorn hens were randomly placed in wire cages when they were 31 weeks old in experiment 1 and 38 weeks old in experiment 2. Sixteen lots with a total of 576 birds in experiment 1 and 18 lots with a total of 216 birds in experiment 2 were fed experimental diets for 140 and 84 days, respectively. Four lots of 36 birds each in experiment 1 and three

TABLE 1

Composition of diets in experiment 1

Ingredient	Crude fiber, %			
	2.05[1]	4.41	6.68	8.79
	%	%	%	%
Yellow corn	69.65	63.45	57.63	54.71
Soybean meal, 49% protein (N × 6.25)	17.94	11.90	5.98	—
Sunflower meal, 33% protein (N × 6.25)	—	10.63	20.88	30.07
Animal fat (tallow)	1.49	3.37	5.00	5.00
Dicalcium phosphate (22% Ca, 18.5% P)	2.49	2.43	2.37	2.31
Limestone	7.52	7.32	7.23	6.98
Sodium chloride	0.25	0.25	0.25	0.25
Trace element premix[2]	0.25	0.25	0.25	0.25
Methionine hydroxy analogue—Ca, 93%	0.34	0.30	0.27	0.24
L-lysine·HCl (98%)	0.07	0.10	0.14	0.19
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Crude protein, %	15	15	15	15
Metabolizable energy, kcal/kg	2970	2970	2970	2970
Total calcium, %	3.50	3.50	3.50	3.50
Available phosphorus, %[3]	0.55	0.55	0.55	0.55
Meth. & Cyst., %	0.80	0.80	0.80	0.80
Lysine, %	0.80	0.80	0.80	0.80
Chemical analysis				
Total fat, %	4.34	6.11	7.66	7.75
Crude fiber, %	2.05	4.41	6.68	8.79
Cholesterol, mg/g	0.47	0.70	1.22	1.26

[1] Standard laying hen diet. [2] The laying hen premix furnished the following amounts of other ingredients per kilogram of feed: retinyl palmitate, gelatin coated, 7177 IU; cholecalciferol, 2205 ICU; vitamin E, 1.1 IU; reboflavin, 3.3 mg; niacin, 22.0 mg; d-pantothenic acid, 6.6 mg; vitamin B-12, 5.5 mg; choline chloride, 441 mg; iron sulfate, 20 mg; copper sulfate, 2 mg. [3] Phosphorus from plant sources is considered to be 30% available to the chick.

lots of 12 each in experiment 2 were fed each experimental diet. Water and feed were given to all birds ad libitum throughout the test periods.

Experimental diets are shown in tables 1 (experiment 1) and 5 (experiment 2). Dietary fiber levels of 2.05, 4.41, 6.68, and 8.79% furnished mainly by sunflower meal, were fed in experiment 1. In experiment 2, alfalfa meal, ground whole oats, sunflower meal, rice mill feed, or wood shavings was added to a corn-soybean meal diet to furnish 2.00% added fiber. Diets in each experiment were formulated to either meet or exceed National Research Council's (19) recommendations for poultry. Natural feed ingredients such as corn, soybean meal, and animal fat were used to formulate the base of the diet since these ingredients are most commonly used in the poultry industry. Feed samples were ether-extracted and dietary fat and cholesterol determinations made (See tables 1 and 5). Proximate analysis of fiber sources were determined by the procedures of Association of Official Agricultural Chemists (20).

Individual blood samples were taken from five hens per lot on 3 consecutive

TABLE 5

Composition of diets in Experiment 2

Ingredient	Source of added fiber					
	Basal	Alfalfa meal	Ground whole oats	Sunflower meal	Rice mill feed	Wood shavings
Yellow corn	72.71	66.12	55.25	70.17	63.14	66.79
Soybean meal 49% protein (N × 6.25)	17.55	16.10	16.33	12.73	18.51	18.61
Alfalfa meal, 17% protein (N × 6.25)	—	7.60	—	—	—	—
Ground whole oats	—	—	18.18	—	—	—
Sunflower meal, 33% protein (N × 6.25)	—	—	—	8.00	—	—
Rice mill feed, 6% protein (N × 6.25)	—	—	—	—	6.25	—
Wood shavings	—	—	—	—	—	3.23
Animal fat (Tallow)	—	1.08	1.00	—	2.31	1.55
Dicalcium phosphate (23% Ca, 18.5% P)	1.94	1.97	1.94	1.92	2.02	1.94
Limestone	7.18	6.42	6.70	6.58	6.65	7.18
Sodium chloride	0.25	0.25	0.25	0.25	0.25	0.25
Trace element premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Methionine hydroxy analogue—Ca, 98%	0.10	0.10	0.10	0.06	0.12	0.10
L-lysine·HCl (98%)	0.02	0.02	—	0.04	—	—
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analysis						
Crude protein, %	15.00	15.00	15.00	15.00	15.00	15.00
Metabolizable energy, kcal/kg	2919.00	2860.00	2853.00	2878.00	2860.00	2862.00
Total calcium, %	3.25	3.25	3.25	3.25	3.25	3.25
Available phosphorus, %[2]	0.45	0.45	0.45	0.45	0.45	0.45
Methionine + Cystine, %	0.60	0.60	0.60	0.60	0.60	0.60
Lysine, %	0.75	0.75	0.75	0.75	0.75	0.75
Chemical Analysis						
Total fat, %	2.96	3.92	4.11	2.87	5.49	4.52
Crude fiber, %	2.09	3.90	3.68	3.90	3.91	4.01
Cholesterol, mg/g	0.47	1.54	0.90	0.49	0.69	0.40

[1] The layer premix furnished the following amounts of other ingredients per kilogram of feed; retinyl palmitate, gelatin coated, 7717 IU; cholecalciferol, 2205 ICU; vitamin E, 1.1 IU; riboflavin, 3.3 mg; niacin, 22.0 mg; d-pantothenic acid, 6.6 mg; vitamin B-12, 5.5 mg; choline chloride, 441 mg; ethoxyquin, 55 mg; manganese, 66.2 mg; zinc, 44 mg; iodine, 1.25 mg; iron sulfate, 20 mg; copper sulfate, 2 mg.
[2] Phosphorus from plant sources is considered to be 30% available to the chick.

TABLE 2

Plasma and liver cholesterol and plasma triglycerides of hens fed various levels of fiber, experimental 1

Crude fiber	Plasma		Liver[1]	
	Cholesterol	Triglycerides	Cholesterol	Weight
%	*mg/100 ml*		*mg/g*	*g*
2.05	158.33±7.21[1,a]	297.65±12.95[a]	2.52±0.11[d]	40.01±2.53[c]
4.49	164.66±7.54[a]	293.63±15.44[a]	2.68±0.14[c]	42.95±1.84[b]
6.68	185.00±9.01[a]	272.65±14.13[b]	3.04±0.21[b]	44.54±2.61[a,b]
8.79	178.33±8.69[a]	245.23±11.49[c]	3.38±0.06[a]	45.61±2.25[a]

[1] Means within a column and without a common superscript letter are significantly different ($P < 0.05$). Mean±SEM for 20 hens.

days in each experiment during the last week of the test. Blood was collected from a total of 20 hens in experiment 1 and 15 hens in experiment 2 for each test group. Plasma cholesterol concentrations were determined by using the Liebermann-Burchard reagent[1,2] consisting of sulfuric acid, acetic acid, and acetic anhydride. Plasma triglyceride levels were determined by the methods described by Pinter et al. (21) and Witter and Whitner (22).

On 3 consecutive days each week, during the last 4 weeks of each experiment, 15 eggs weighing 59 ± 2 g and 15 eggs weighing 64 ± 2 g were randomly collected from hens fed each test diet in each experiment. Eggs from each group were broken and yolks were cleaned thoroughly with cheesecloth. All yolks from each test group were pooled and yolk cholesterol determined. Yolk samples were extracted by the method of Folch et al. (23). Yolk cholesterol was determined with the use of the Liebermann-Buchard reagent.[1,2]

Statistical examination of the data was performed using the analysis of variance (24). Duncan's New Multiple Range Test (25) was used to determine significant differences between means. All statements of significance refer to the 5% level of probability.

RESULTS AND DISCUSSION

Experiment 1. Results of experiment 1 are shown in tables 2 to 4. No significant differences in plasma cholesterol (table 2) were found due to dietary fiber level. How-

[1] Hycel, Inc., P.O. Box 36329, Houston, Texas.
[2] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

TABLE 3

Yolk weight and egg yolk cholesterol concentrations of hens receiving various crude fiber levels, experiment 1

Crude fiber	Yolk wt.			Yolk cholesterol			Yolk cholesterol		
	Egg wt., g			Egg wt., g			Egg wt., g		
	59±2	64±2	Mean[1]	59±2	64±2	Mean[1]	59±2	64±2	Mean[1]
%	*g*			*mg/g*			*mg*		
2.05	19.09	21.02	20.06±1.06[2]	14.42	93.92	14.17±0.70[a]	275.23	292.53	283.88±18.34[d]
4.41	19.10	20.37	19.74±0.84	12.75	12.75	12.75±0.64[b]	262.66	280.15	271.41±13.33[c]
6.68	18.46	20.54	19.56±0.89	12.82	13.82	13.32± .59[a]	236.85	271.95	254.40±14.51[b]
8.79	18.84	20.29	19.48±1.13	12.23	12.92	12.62±0.75[a]	230.14	262.19	246.16±16.55[a]
Mean[1]	18.87[b]	20.34[a]		13.33[a]	13.60[a]		251.72[a]	276.71[b]	

[1] Means within a column or row grouping and without a common superscript letter are significantly different ($P < 0.05$). Mean ±SEM. [2] No significant differences ($P < 0.05$) in yolk weights were found due to dietary treatment. Mean±SEM. [3] Eggs of equal weights were used for cholesterol concentration determinations.

TABLE 4

Effect of fiber level on egg production and weight, experiment 1

Crude fiber	Test results[1]		
	Hen-day lay	Avg. egg wt.	Feed consumed
%	%	g	g/hen/day
2.05	78.26 ± 3.99[a]	59.6 ± 2.51[a]	78.19 ± 2.01[a]
4.41	77.08 ± 4.05[a]	59.4 ± 1.89[a]	87.53 ± 3.95[b]
6.68	77.08 ± 2.13[a]	59.2 ± 2.36[a]	91.36 ± 3.41[ab]
8.79	77.17 ± 3.15[a]	59.1 ± 1.97[a]	95.66 ± 3.25[b]

[1] Means within a column and without a common superscript letter are significantly different ($P < 0.05$). Mean ± SEM for 144 hens.

ever, plasma triglycerides decreased as hens were fed diets with increasing dietary fiber levels ($r = -0.95$). Although total dietary fat (table 1) increased as dietary fiber increased in order to equate metabolizable energy among diets, plasma triglycerides decreased as dietary fat and fiber increased (see table 2). Liver cholesterol per g of liver and liver weights increased (table 2) as both fiber and animal fat increased in laying hen diets. Either fiber or animal fat might have caused the increased liver weights. No conclusion was reached as to the cause of liver weight increases, since liver lipids were not determined.

Egg weights were separated into two distinct egg weight groups weighing 59 ± 2 or 64 ± 2 g and egg yolk cholesterol was determined on each group. Eggs of similar weights were taken in order to delete any possible interaction of egg weight and yolk cholesterol. Yolk cholesterol and weights are shown on table 3. Egg yolk weights numerically decreased as fiber was increased in laying hen diets; however, these values were not significantly different.

Milligrams yolk cholesterol per g of yolk and mg cholesterol per yolk decreased as hens were fed increasing dietary fiber levels. Total cholesterol per yolk decreased 4.39, 10.38, and 13.29% by feeding crude dietary fiber levels of 4.41, 6.68, and 8.79%, respectively, to hens as compared to a corn-soybean meal basal diet containing 2.05% crude fiber. Although dietary fat increased as dietary fiber increased (table 1), the conclusion is reached that only dietary fiber influenced yolk cholesterol, since Miller and Katsoulis (26) found no significant differences in either blood serum or egg yolk cholesterol concentrations with increasing dietary animal fat.

No significant differences in either egg production or egg weight (table 4) were found due to diet variation. However, feed consumption increased as dietary fiber increased in the diet of laying hens.

Experiment 2. Results of experiment 2 are shown in tables 6 to 8. Plasma cholesterol (see table 6) was significantly lower when laying hens were fed diets containing alfalfa meal, wood shavings, oats, or rice mill feed when compared to laying hens fed a basal diet or sunflower meal. Plasma triglycerides significantly increased when diets containing oats were fed to laying hens as compared to laying hens fed the corn and soybean meal basal diet.

No significant differences in yolk weights (table 7) were found among diets fed.

TABLE 6

Plasma and liver cholesterol and plasma triglycerides of hens fed various fiber sources, experiment 2

Fiber source	Plasma		Liver[1]	
	Cholesterol	Triglycerides	Cholesterol	Weight
	mg/100 ml		*mg/g*	*g*
None	128.97 ± 10.40[1,d]	319.41 ± 14.13[cd]	1.65 ± 0.08[ab]	44.07 ± 2.21[a]
Alfalfa meal	55.98 ± 5.53[a]	346.80 ± 18.55[bc]	1.94 ± 0.06[d]	45.01 ± 2.35[a]
Ground whole oats	87.99 ± 9.14[bc]	373.49 ± 21.31[c]	1.71 ± 0.11[bc]	46.24 ± 1.94[a]
Sunflower meal	141.03 ± 11.59[d]	335.57 ± 10.21[cb]	1.60 ± 0.05[ab]	58.24 ± 2.29[b]
Rice mill feed	98.20 ± 10.13[c]	309.76 ± 12.14[a]	1.78 ± 0.12[c]	52.09 ± 2.61[b]
Wood shavings	67.28 ± 7.25[ab]	326.70 ± 16.91[ab]	1.55 ± 0.10[a]	53.76 ± 1.99[ab]

[1] Means within a column and without a common superscript letter are significantly different ($P < 0.05$). Mean ± SEM for 15 hens.

TABLE 7

Egg yolk cholesterol concentrations of hens receiving various crude fiber sources, experiment 8

Fiber source	Yolk wt.			Yolk cholesterol			Yolk cholesterol		
	Egg wt., g			Egg wt., g[2]			Egg wt., g		
	59 ±2	64 ±2	Mean[1]	59 ±2	64 ±2	Mean[1]	59 ±2	64 ±2	Mean[1]
	g			mg/g			mg		
None	18.22	20.12	19.17 ±0.85	12.92	14.78	13.80 ±0.84[a]	235.47	295.32	265.40 ±14.47[a]
Alfalfa meal	18.20	20.01	19.10 ±1.15	13.06	13.70	13.38 ±0.71[a]	237.76	274.15	255.95 ±12.51[a]
Ground whole oats	18.09	19.85	18.97 ±0.94	11.95	13.08	12.50 ±0.65[a]	216.13	259.20	237.66 ±16.16[a]
Sunflower meal	18.31	19.50	18.90 ±0.68	12.78	12.75	12.27 ±0.77[a]	234.19	268.12	251.16 ±14.91[b]
Rice mill feed	18.49	20.09	19.29 ±1.08	12.78	13.21	12.99 ±0.94[b]	236.36	266.44	250.55 ±12.99[b]
Wood shavings	18.30	20.04	19.17 ±1.11	11.95	12.79	12.37 ±0.53[a]	218.63	256.25	237.44 ±16.18[a]
Mean[1]	18.27[b]	19.94[a]		12.57[a]	13.55[b]		229.74[a]	269.74[b]	

[1] No significant differences ($P < 0.05$) in yolk weights were found due to dietary treatment. Mean ±SEM. [2] Eggs of equal weight were used for cholesterol concentration determinations. [3] Means within a column or row grouping and without a common superscript letter are significantly different ($P < 0.05$).

Yolk cholesterol of laying hens was decreased by feeding either oats or wood shavings as fiber sources when compared to either the basal or diets containing alfalfa meal, sunflower meal, or rice mill feed.

Husseini et al. (9) found a small reduction in yolk cholesterol by feeding a diet containing 15% ground oats and 3% vegetable oil; however, this reduction was not significantly different. Wood et al. (18) found a marked elevation in yolk cholesterol with 10% cholesterol in a chick's diet, and 10% corn oil in the diet enhanced this elevation. There is a possibility that the effects of oats on yolk cholesterol reported by Husseini et al. (9) were not as great as reported in this study because of the addition of 3% vegetable oil in their diets along with 15% oats. Wood et al. (18) reported that vegetable oil increased yolk cholesterol, therefore, the depression of yolk cholesterol which might have occurred with oats in the study of Husseini et al. (9) was probably negated by the addition of vegetable oil. Our results do indicate that oats reduced yolk cholesterol of laying hens by about 10% (table 7), when compared to a corn-soybean basal diet.

Milligrams cholesterol per g of liver increased (table 6) significantly when laying hens were fed alfalfa meal as compared to hens fed the basal, oats, sunflower meal, rice mill feed, or wood shavings. Total liver cholesterol increased significantly when hens were fed either alfalfa meal or rice mill feed as compared to hens fed the basal diet.

Egg production and egg weight were not

TABLE 8

Effect of fiber source on egg production and weights, experiment 9

Fiber source	Test results[1]		
	Hen-day lay	Avg. egg wt.	Feed consumed
	%	g	g/hen/day
None	79.38 ±4.05[a]	60.0 ±3.01[a]	80.58 ±4.55[a]
Alfalfa meal	78.81 ±3.51[a]	59.5 ±2.29[a]	87.93 ±4.21[b]
Ground whole oats	78.50 ±3.84[a]	60.1 ±2.85[a]	89.21 ±3.89[b]
Sunflower meal	78.95 ±4.16[a]	59.8 ±2.57[a]	82.60 ±4.05[b]
Rice mill feed	78.23 ±4.17[a]	59.4 ±2.88[a]	89.09 ±3.63[b]
Wood shavings	79.06 ±3.26[a]	60.1 ±2.53[a]	86.32 ±3.51[b]

[1] Means within a column and without a common superscript letter are significantly different ($P < 0.05$). Mean ±SEM for 36 hens.

affected (table 8) by diet, although feed consumption increased in diets containing additional fiber sources. Data reported in this study indicate that feeding either oats or wood shavings up to 2.00% added fiber reduced yolk cholesterol without adversely affecting either egg production or egg weight. Furthermore, yolk cholesterol was reduced linearly with increasing levels of dietary fiber, using sunflower meal as the fiber source, without influencing egg production or egg weight.

LITERATURE CITED

1. Balmer, J. & Zilversmit, B. D. (1974) Effects of dietary roughage on cholesterol absorption, cholesterol turnover, and steroid excretion in the rat. J. Nutr. *104*, 1319–1328.
2. Trowell, H. (1972) Ischemic heart disease and dietary fiber. Am. J. Clin. Nutr. *25*, 926–932.
3. Kritchevsky, D., Moyer, A. W., Tesar, W. C., Logan, J. B., Brown, R. A., Davies, M. C. & Cox, H. R. (1954) Effect of cholesterol vehicle in experimental atherosclerosis. Am. J. Physiol. *178*, 30–32.
4. Tsai, A. C., Elias, J., Kelley, J. J., Lin, R. S. C. & Robson, J. R. K. (1976) Influence of certain dietary fibers on serum and tissue cholesterol levels in rats. J. Nutr. *106*, 118–123.
5. Fahrenbach, M. J., Riccardi, B. A. & Grant, W. C. (1966) Hypocholesterolemic activity of mucilaginous polysaccharides in White Leghorn cockerels. Proc. Soc. Exp. Biol. Med. *123*, 321–326.
6. Fisher, H. & Griminger, P. (1967) Cholesterol-lowering effects of certain grains and of oat fractions in the chick. Proc. Soc. Exp. Biol. Med. *126*, 108–111.
7. Simpson, C. F. & Harms, R. H. (1969) Influence of oat fractions on diethylstilbesterol-induced aortic ruptures of turkeys. Poultry Sci. *48*, 1757–1761.
8. Menge, H., Littlefield, L. H., Frobish, L. T. & Weinland, B. T. (1974) Effect of cellulose and cholesterol on blood and yolk lipids and reproductive efficiency of the hen. J. Nutr. *104*, 1554–1558.
9. Husseini, M. D., Krueger, W. F., Fanguy, R. C. & Bradley, J. W. (1976) Blood serum and egg yolk cholesterol in hens as influenced by wheat middlings and oats in the diet. Poultry Sci. *55*, 1506.
10. Keshavarz, L. (1976) The influence of turmeric and curcumin on cholesterol concentration of eggs and tissues. Poultry Sci. *55*, 1077–1083.
11. Edwards, H. M., Jr., Marion, J. E. & Driggers, J. C. (1962) Serum and egg cholesterol levels in mature hens as influenced by dietary protein and fat changes. Poultry Sci. *41*, 713–717.
12. Palafox, A. L. (1968) Effect of age, energy source, and concentration on yolk lipids and cholesterol. Poultry Sci. *47*, 1705.
13. Dua, P. N., Dilworth, B. C., Day, E. J. & Hill, J. E. (1967) Effect of dietary vitamin A and cholesterol on cholesterol and carotenoid content of plasma and egg yolk. Poultry Sci. *46*, 530–531.
14. Nockels, C. F. (1973) The influence of feeding ascorbic acid and sulfate on egg production and on cholesterol content of certain tissues of the hen. Poultry Sci. *52*, 373–378.
15. Hafez, Y. S. M. & Kratzer, F. H. (1976) The effect of pharmacological levels of dietary vanadium on the egg production, shell thickness, and egg yolk cholesterol in laying hens and coturnix. Poultry Sci. *55*, 923–928.
16. Turk, D. E. & Barnett, B. D. (1972) Diet and egg cholesterol content. Poultry Sci. *51*, 1881.
17. Story, J. A. & Kritchevsky, D. (1976) Comparison of the binding of various bile acids and bile salts in vitro by several types of fiber. J. Nutr. *106*, 1292–1294.
18. Wood, J. D., Biely, J. & Topliff, J. E. (1961) The effect of diet, age, and sex on cholesterol metabolism in White Leghorn chickens. Can. J. Biochem. Physiol. *39*, 1705–1715.
19. National Research Council (1971) Nutrient requirements of domestic animals. No. 1. Nutrient requirements of poultry.
20. Association of Official Agricultural Chemists (1965) Official Methods of Analysis, 10th Ed., Washington, D.C.
21. Pinter, J. K., Hayashi, J. A. & Watson, J. A. (1967) Enzymatic assay of glycerol, dihydroxyacetone, and glyceraldehyde. Arch. Biochem. Biophys. *21*, 404.
22. Wittar, R. F. & Whitnar, V. S. (1972) Determination of serum triglycerides. Blood Lipids and Lipoproteins Quantitation, Composition and Metabolism, John Wiley and Sons, Inc.
23. Folch, J., Lees, M. & Stanley, G. H. S. (1957) A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. *226*, 497–509.
24. Steel, T. G. D. & Torrie, T. H. (1960) Principles and procedures of statistics. McGraw-Hill Book Company, Inc., New York, N.Y.
25. Duncan, D. B. (1955) Multiple range and multiple F tests. Biometrics *11*, 1–42.
26. Miller, R. I. & Katsoulis (1974) Influence of high fat diet on caged layers. Poultry Sci. *53*, 1955.